SUMMARY

India has a rich source of medicinal plants, herbs and shrubs, which includes about more than 1500 species and has a vast geographical area with great potential abilities for Ayurvedic, charaka, Siddha, Unani traditional medicines but only very few plants were explored pharmacologically and chemically for their potential therapeutic value. People have used many plant sources for the cure of diversified diseases for thousands of years. According to World Health Organization, most of the population still relies on traditional medicines for their disease treatment, since many of them cannot afford the products of pharmaceuticals, because of their side effects. Many rural and some of the urban areas of many developing countries still depend on traditional medicine for their health care needs. These traditional medicines are more safe and cheaper than synthetic medicine. Some medicinal plants show high effect only when they are used in proper therapeutic doses.

The immune responses can be modulated by the administration of different immunomodulatory agents. Immunomodulation can of two type namely, immunosuppression and immunostimulation. While immunostimulation is a mechanism, which directly enhances one or more specific functions of the immune system or modifies the components of immunoregulatory network of immune response to meet its objective.

The present study focuses on the plant sources from north Andhra Pradesh, which involve in the modulation of immune response.

*Terminalia chebula, Punica granatum, Syzygium jambolanum, Aegle marmelos, Nyctanthes arbor-tristis, Annona squamosa, Acalypha indica, Momordica charantia, Tylophora indica* and *seeds of Zea mays* were selected as plant sources for studying immunomodulatory activity.

The leaves of these plants and seeds of *Zea mays* were dried and extracted using aqueous and solvent extracts and were concentrated.

The lyophilized extracts were tested on animal models. 1mg of extract was injected along with antigen (OVAAlbumin)on days 0, 21 and 42. The mice were bled on days 14, 28 and 49 for
determining humoral immune response by ELISA. It was observed that five plants TC, NA, TI, PG and AI have shown immunostimulant activity on anti OVA IgG, anti OVA IgM and anti OVA IgA for primary, secondary and tertiary immune responses and no response on IgE. Further these plants were purified by solvent fractionization (ethanol, ethyl acetate, chloroform). These results shown that out of all the solvent fractions of five plants ethanolic extract *Terminalia chebula* and further studies were carried out with ethanolic extract of *Terminalia chebula* (TCE). Further TCE was subjected to phytochemical analysis. The results of TCE have shown positive for the presence of phenols, flavanoids, triterpenes, and glycosides.

TCE is further tested on spleen cells of mice to determine cell mediated immunity. The spleen cells were isolated from mice and MTT assay was carried to determine the T- cell proliferation. The results indicated that TCE have shown high activity when compared to control. TCE is then subjected to silica gel column chromatography.

TCE fraction was subjected to purification by silica gel column chromatography using solvents benzene and ethyl alcohol (100:90:20; 80:20; 70:30). A total of 226 fractions were collected from the column. Alike fractions were pooled by UV spectrophotometer and the homogeneity of the fractions were detected by Thin Layer chromatography (TLC).

The fractions obtained from silica gel chromatography were S1 to S16 which were tested on animal model for both humoral and cell mediated response and phytochemical studies carried out. The results indicate that S12 fraction shown highest stimulant activity and it is a phenol compound.

This S12 fraction was analyzed by using advanced analytical techniques. UV absorption spectrum at a range of 200nm-700nm was scanned and the compound showed absorption at 254 nm indicating it a phenol. The molecular weight of the compound is determined by LC-MS and is 194.16. The structure of the compound has been analyzed by Infra red spectrum and by carbon and hydrogen NMR spectrum. The predicted structure of the compound has been obtained and was identified as 4-Hydroxy-3-methoxycinnamic acid, which is an immunomodulant on humoral and cell mediated immunity showing immunostimulant activity.

There are plenty of reports on different plant sources showing different properties which are therapeutically useful and advantageous. There are many plant sources identified for having
immunomodulatory property. Different herbal formulations with different combinations and various fractions were used for establishing the property. But no specific mechanism of action for these compounds was clearly explained. Most of the identified and isolated compounds deal with the immunosuppressive property. There is very little to know about the immunostimulatory compounds and their mechanism of their action. The experiments in the present investigation strongly confirm the presence of immunostimulatory compounds in the ethanol fraction of the extract.

In brief, 4-Hydroxy-3-methoxycinnamic acid compound was isolated from TC and it has found to be stimulating on IgG, IgM and IgA and without any response on IgE. It was immunomodulating antigen induced T-cell response. The adjuvant property of this compound may be extended to practical scenarios where boosting of immune response is needed in the case of pure antigen.